PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
2022264PC//HP/MIK		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI2003/000888	19.11.2003	19.11.2002
International Patent Classification (IPC) or national classification and IPC		
C12Q 1/68 // C12N 15/11		
Applicant		
Mobidiag Oy et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 15 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☒ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.06.2004	23.02.2005
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Sara Nilsson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000888

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:
pages 1-16, 18-30 as originally filed/furnished
pages* 17 received by this Authority on 25-10-2004
pages* _____ received by this Authority on _____

☒ the claims:
pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 31-33 received by this Authority on 25-10-2004
pages* _____ received by this Authority on _____

☒ the drawings:
pages 1-3 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☒ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

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Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority is considered valid. Document Fukushima M. et al, "Detection and identification of mycobacterium species isolates by DNA microarray", Journal of clinical microbiology, 2003, pp. 2605-2615 is therefore not considered herein.

Box No. IV Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

☐ complied with.

☒ not complied with for the following reasons:

Invention 1): Claims 1-14 and 21 directed to a method for detecting and identifying bacterial species using specific broad-range primers.
Inventions 2-13): Claims 15-20 (claims 15-18 partially, the parts relating to probes specific for gyrB), directed to oligonucleotide sequences that hybridize to hyper-variable species-specific genomic regions.
Invention 14): Claims 15-20 (claims 15-18 partially, the parts relating to probes specific for parE, directed to oligonucleotide sequences that hybridize to hyper-variable species-specific genomic regions.

Claims 1-14 and 21 (invention 1) relate to the problem of detecting and identifying bacterial species. This problem is solved by using the broad-range primers SEQ ID NOs: 76 and 77 together with species-specific hybridization probes. The special technical features over the general state of the art are the specific broad-range primers used in the method. The primers hybridize with sequences originating from conserved regions of genes encoding topoisomerases.

The closest prior art in relation to inventions 2-14 has been

.../...

4. Consequently, this report has been established in respect of the following parts of the international application:

☒ all parts.

☐ the parts relating to claims Nos. _____

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box IV

sequences from e.g. Escherichia coli, Staphylococcus aureus and Streptococcus pneumoniae are disclosed but no examples showing probes from these regions are shown. It is stated that hybridization of an amplified region of e.g. the gyrB or parE gene to species-specific probes fixed to a surface, can be employed for genotypic classification of bacteria. See p. 2 paragraph 3, p. 5 paragraphs 1 and 3, p. 6 and p. 10 claim 4.
D2 discloses the use of sequences originating from the gyrB gene as probes able to discriminate between bacterial species. See abstract and [0020]-[0023] and [0039]-[0040].
D3 shows species-specific gyrB probes. See [0010] and [0018]-[0021].
D4 shows a method for detection of slow growing mycobacteria species using parts of the gyrB or complementary sequences thereof as probes. See [0018], [0034], [0035], [0043], [0048], claims 2 and 6.

Thus, sequences originating from or hybridizing to the gyrB and parE genes from different bacterial species are known to be used as the basis for species-specific hybridization probes, and such probes originating from or hybridizing to the gyrB are disclosed in the cited prior art. The fact that the probes disclosed in the present application relate to bacteria causing infections in the respiratory tract is not considered a special technical feature in the sense of Rule 13.2 PCT since it is obvious to the skilled person, given what is disclosed in the prior art, that different bacterial species can be detected using the gyrB or parE genes and since Streptococcus pneumoniae (a bacterium causing respiratory tract infections) is mentioned in D1 and since bacteria causing respiratory tract infections are discussed in D4.

Inventions 2-13:
From a comparison of the disclosure of prior art and the technical features of Inventions 2-13 (claims 15-18 partially, the parts relating to probes specific for gyrB, and 19-20), the following technical features can be seen to make a contribution over this prior art:
Sequences that can be used as species-specific probes with specificity for the gyrB gene from 12 different bacterial species are disclosed (the bacterial species are shown in Table 4A). Probes specific for each bacterial species represent one invention, hence 12 inventions are disclosed. These technical features are hence considered as special technical features in the sense of Rule 13.2 PCT. .../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box IV

From these special technical features the objective problem to be solved by inventions 2-12 respectively, can be construed as: providing sequences that can be used as species-specific probes with specificity for the gyrB gene from each of the 12 different bacterial species shown in table 4A.

Invention 14:
From a comparison of the disclosure of prior art and the technical features of Invention 14 (claims 15-18 partially, the parts relating to probes specific for parE, and 19-20), the following technical features can be seen to make a contribution over this prior art:
Sequences that can be used as species-specific probes with specificity for the parE gene from certain bacterial species (the bacterial species are disclosed in table 4B). These features are hence considered as special technical features in the sense of Rule 13.2 PCT.
From these special technical features the objective problem to be solved by the second invention can be construed as: providing sequences that can be used as species-specific probes with specificity for the parE gene from the specified bacterial species.

The above analysis shows that the special technical features of inventions 1-14 are neither the same nor corresponding.

Consequently, neither the objective problem underlying the subjects of the 14 claimed inventions, nor their solutions defined by the special technical features, allow for a relationship to be established between the said inventions, which involves a single general inventive concept.

In conclusion, therefore, the 14 groups of claims are not linked by same or corresponding special technical features and define different inventions not linked by a single general inventive concept.
The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT.

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-19	YES
	Claims		NO
Inventive step (IS)	Claims	1-14, 19	YES
	Claims	15-18	NO
Industrial applicability (IA)	Claims	1-19	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:
D1) WO0136683 A2
D2) Yamamoto S et al: "PCR amplification and direct sequencing of gyrB genes with universal primers and their application to the detection and taxonomic analysis of pseudomonas putida strains", Applied and environmental microbiology, 1995, vol. 61, no. 3, pp. 1104-1109
D3) US5645994 A
D4) EP0935003 A2
D5) GB2364054 A
D6) WO0061796 A1
D7) EP0965636 A1
D8) EP1098003 A2
D9) JP2001245677 & WPI abstract
D10) JP2000060570 & WPI abstract
D11) US6087104 A
D12) WO9950458 A2
D13) WO0052203 A2
D14) Fukushima M. Et al: "Phylogenetic analysis of Salmonella, Shigella and Escherichia coli strains on the basis of the gyrB gene sequence", Journal of clinical microbiology, 2002, vol. 40 no. 8 pp. 2779-2785
D15) Huang W. M., "Bacterial diversity based on typeII DNA topoisomerase genes", Annual review of genetics, 1996, vol. 30 pp. 79-107

D1 shows methods and compositions useful for rapid identification of microorganisms. It is stated that by choosing PCR primers from among sequences highly conserved in a number of bacteria, one set of PCR primers or a set of degenerate primers can be used for amplification of an unknown

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

sample for revealing its identity. Hybridization probes will be chosen from the less conserved sequences flanked by the PCR primers, each probe having an exact match in a particular pathogen. A number of gram-negative bacteria which can be detected are mentioned. It is stated that Type I and Type II topoisomerases are examples of highly conserved genes in prokaryotes and that segments of these genes may be suitable for the purpose of the method. Conserved coding sequences are selected such that they are highly conserved at both ends of an operationally defined gene fragment and more divergent in the intervening coding sequence. It is stated that coding sequences such as topoisomerases can be used as signature probes since degenerate primers can be designed to amplify these sequences. Arrays of oligonucleotide probes having a sequence corresponding to a species specific target sequence wherein the species specific target sequence is flanked by sequences conserved across a plurality of organisms are claimed as well as a kit comprising primers and probes. See p. 2 lines 9-19, p. 7 line 6-p. 8 line 12, p. 9 lines 12-20, p. 16 line 28-p. 18 line 29, p. 20 line 3- p. 22 line 14, p. 44-45; claims 12, 16 and 20.

D2 shows universal degenerate PCR-primers for the amplification of gyrB genes. Consensus amino acid sequences of gyrases from E. coli, P. Putida and B. Subtilis are used to design the primers. It is stated that DNA probes in combination with PCR can be used to detect bacteria and that the resolution of the detection of specific microorganims is highly dependent on the specificity of the probes or PCR primer sets used. The gyrB genes are chosen as targets of highly specific probes. It is stated that the method for amplification and sequencing of gyrB genes reported may be useful for the rapid development of species specific probes, for taxonomic analysis and for the identification of bacteria. See p. 1104 left column-right column paragraph 1 and p. 1109 left column paragraph 2.

D3 shows a method of identifying species in a sample based on pairs of consensus amino acid segments which flank variable

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

amino acid segments of typeII DNA topoisomerases. The universal primers shown are derived from DNA sequences coding for amino acid consensus sequences of type II topoisomerases. The universal primers are used to amplify DNA segments coding for the variable signature amino acid sequences, where after the amplified segments are sequenced and matched against a database. It is stated that the parE amino acid sequence can be sufficiently homologous to the gyrB subunit to be useful in the method. ParE and gyrB share consensus regions. See col. 5 lines 26-37, col. 6 lines 28-39, col. 7 lines 19-30, col. 8 lines 21-27 and col. 14 lines 8-18.

D4 shows a method for identification and detection of organisms using the sequences of their genes encoding the B unit of the DNA gyrase. Primers which amplify gyrB but not parE are shown. See abstract, p. 4 lines 8-11, [0007], [0009]-[0013], [0037].

D5 shows a method for identifying regions of bacterial polynucleotide sequences associated with quinolone resistance. Degenerate primers with a degenerate half and a constant half are disclosed. The primers are generated by analysing gyrase and topoisomerase IV sequences from a number of bacterial species. See p. 2 paragraph 2-p. 3 paragraph 1, p. 4 paragraphs 6-8, p. 8 paragraph 4.

D6 discusses the use of fragments of sequences complementary to sequences originating form e.g. gyrB or parE genes as species-specific probes. The gyrB and parE sequences from e.g. E. Coli, S. Aureus and S. Pneumoniae are disclosed. Criteria for genes suitable for the identification of bacteria are disclosed. It is stated that hybridization of an amplified region of e.g. the gyrB or parE gene to species-specific probes fixed to a surface, can be employed for genotypic classification of bacteria. See p. 2 paragraph 3, p. 5 paragraphs 1 and 3, p. 6, p. 10 claim 4.

D7 shows a method of detecting V. Parahaemolyticus by amplifying a part of the DNA gyrase subunit B gene. The method is used to differentiate V. Parahaemolyticus from other

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Vibrio species. Primers for amplifying the gene fragments are disclosed. Probes which are specific for the internal consensus sequence of the primer amplification product are disclosed. See [0010], [0018]-[0021].

D8 shows a method for identification and detection of mycobacteria utilizing characteristic nucleotide sequences which are present in the gyrB gene. DNA microarray techniques are discussed and the sequences can be used as primers or as probes. See p. 3 lines 50-52, [0018], [0027], [0035] lines 22-25, [0037], [0048], p. 84 claim 6.

D9 shows means for discriminating bacterial species belonging to the Shigella or Salmonella genus by using sequences of domains of the gyrB gene. The sequences can be used as primers or probes. See abstract, [0020]-[0023], [0039]-[0040].

D10 shows nucleic acid fragments useful for detecting microbes. Parts if the gyrB gene can be detected using the disclosed sequences as probes specific to different bacterial species. See abstract, [0022], [0024].

D11 shows species specific sequences that can be used in determining the presence or absence of bacterial species by e.g. using the sequences as probes. The sequences originate from the gyrB gene. See col. 5 lines 31-42, col. 6 lines 1-21.

D12 shows oligonucleotide probes for detecting different bacterial species. Unique gyrA coding regions permit the development of probes specific for eight different species. See p. 1 lines 25-29, p. 4 lines 1-10, p. 20 lines 8-34.

D13 shows a method for identifying bacteria in a sample, the method comprising amplifying a portion of the 23S rDNA present in the sample using a degenerate primer set and testing the resulting amplicon by hybridization to one or more oligonucleotide probes designed to identify one or more bacteria likely to be present in the sample. See abstract, p. 2 lines 1-11, p. 2 line 27-p. 3 line 8.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D14 shows that the gyrB region can be useful when analyzing the phylogenetic relationships of bacteria. It is stated that the sequence of the gyrB gene can be used for classification of closely related bacterial species.

D15 shows that highly conserved motifs in gyrB and parE genes provide a rationale for the design of universal primers to use in PCR for bacterial diversity studies. See the abstract and p. 84-85.

The present application relates to nucleic acid probes and broad-range primers. The nucleic acid probes originate from hyper-variable regions situated near the conserved sequences of topoisomerase genes of infection-causing bacteria. The broad-range primers originate from the conserved regions of topoisomerase genes. The problem to be solved is providing tools and methods useful in bacterial diagnostics, the tools and methods being sensitive, effective, species-specific, and being capable of identifying only the desired bacterial species. The methods are faster than previously known methods e.g. since the PCR product amplified is short.

D1 is considered to represent the closest prior art. D1 shows a method for detecting and identifying bacteria by amplifying genes encoding e.g. topoisomerases and contacting the amplified DNA with species-specific probes.
The difference between the invention according to claim 1 and D1 is the specific degenerate primers SEQ ID NOs 76 and 77 used in claim 1. In D1, no specific primers or probes are disclosed, but it is stated that genes encoding topoisomerases can be used in the method which includes primers and probes. Examples wherein genes encoding topoisomerases are used for amplification and hybridization are not experimentally shown, but the use of genes encoding topoisomerases in the method disclosed, is suggested in the description of D1.
The effect achieved by the use of primers 76 and 77 seems to be a sensitive and rapid analysis (the gene product amplified is short).

Thus, the problem to be solved in view of D1 is the provision of a method for detecting and identifying bacterial species using degenerate primers which provides rapid and sensitive

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In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

amplification.

A general statement, as in D1, that conserved sequences of topoisomerase type I and II can be used as primers for the detection of microorganisms does not without question mean that this is the case when clinical samples are to be analyzed. Since the unpredictability is high, the development of a specific and sensitive diagnostic method for detecting and identifying bacterial species from a clinical sample is not considered as a routine procedure for a skilled person, even though specific primer pairs originating from conserved sequences of topoisomerases are disclosed in D2-D5. For example, the effects of the normal flora on the analysis cannot be predicted due to the great number of different bacteria belonging to the human normal flora. When the detection and identification is based on conserved genes present in both eukaryotic and prokaryotic organisms (as in the present application) the effect is more unpredictable.

There are statements in D1 directing a skilled person away from the present invention: Topoisomerases are mentioned but no examples are given (whereas other genes are exemplified). Type I and type II topoisomerases are mentioned as less preferred (see p. 20 lines 3-10, especially lines 9-10) and as not fulfilling the requirements of a conserved sequence of the invention in D1. The example specifically mentioned in D1, FtsK, is conserved only in eubacteria and not in eukaryotes.

Additionally, D1 discourages the skilled person from using universal or broad range primers as in the present application. The "universal" primers actually used in the invention are a mixture of related primers, this reduces the degeneracy of the primer. The degeneracy of a conventional degenerate primer is stated to be too high to be useful.

Consequently, the skilled person, faced with the problem mentioned above (i.e. providing broad range primers for a rapid and sensitive amplification) is discouraged by D1 from starting the design such primers on the basis of topoisomerase sequences.
Documents D2-D5 which disclose primers that originate from conserved sequences of topoisomerases all date before D1. None of the documents disclose results obtained with clinical samples.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Therefore, the invention according to claims 1-14 and 19 is novel and is considered involving an inventive step. The invention according to claims 1-14 and 19 is industrially applicable.
The same holds true if starting from D3; clinical samples are not analyzed and the sequences SEQ ID NOs: 76 and 77 are not provided.

Document D7 is considered to represent the closest prior art in relation to claim 15. D7 discloses the use of fragments of sequences complementary to sequences originating form gyrB genes as species-specific probes. The difference between the invention according to claim 15 and D7 are the specific sequences 1-69 disclosed in claim 15. No unexpected technical effects are shown by the provision of sequences 1-69.
Therefore, the problem to be solved is the provision of alternative species specific probes for the gyrB or parE genes. Providing alternative species-specific probes hybridizing to a sequence (the gyrB or parE sequence) is an obvious measure to the skilled person.
Even if the gyrB or parE sequences of some of the bacteria included in Tables 4 and 5 are not available in public sequence databases, obtaining such sequences must be considered a routine procedure. Testing the specificity and selectivity of probes to identify working ones, is an obvious measure to the skilled person. Consequently, the invention according to claims 15-18 is considered not to involve an inventive step. The same argumentation can be made starting with either one of documents D6 and D8-D11, which all show the use of hypervariable sequences situated near the conserved regions of topoisomerase genes as species-specific probes. The use of sequences complementary to parE as species-specific probes is disclosed in D6, and it is known to the skilled person that this gene is related to gyrB and thus equally is suitable for species-specific probes.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The expression "sequences identified with..." used in present claims 1, 5, 11, 14-17 and 21 is somewhat unclear and misleading. The expression gives the impression that the claims do not actually refer to the sequences named SEQ ID NO: 76, 77 or 1-69, but to sequences identified with those sequences. According to the description, the claims refer to the sequences SEQ ID NO: 76, 77 or 1-69, which thus are supported by the description. No support is given to sequences in some way identified with the sequences SEQ ID NO: 76, 77 or 1-69. The use of the expression above leads to a lack of clarity within the meaning of Art. 6 PCT.

There seems to be an inconsistency in the way that SEQ ID NO: 77 is described. In the sequence-listing it is stated that "r" is "a" or "t", but in the description, "r" is indicated as "a" or "g". This leads to a lack of clarity within the meaning of Art. 6 PCT since it is not clear which sequence is intended.

These primers functioned therefore as broad-range primers for bacteria. Sensitivity of the primers varied, and the primer pair with the highest sensitivity was used for studying the clinical specimens (otitis media samples). However, it was found out that this primer pair was not sufficiently sensitive, because it was not able to amplify bacterial DNA from clinical specimens that contained large amounts of human DNA.

For this reason new primer pairs that varied in terms of degeneration were synthesized (ordered from Sigma-Genosys, England, www.sigma-genosys.co.uk). The specificity and the sensitivity were studied by the previously described method, both with pure bacterial DNA and with DNA isolated from clinical specimens (Table 5). A functioning primer pair was the mixture of primers, which contains the sequences

CGTCCWGGKATGTAYATHGG (SEQ. ID. NR: 76) and
CCHACRCCRTGWAAWCCDCC (SEQ. ID. NR: 77),
which were named as gB1F (forward primer mixture) and gB2R (reverse primer mixture), respectively (Table 1), wherein
W represents base A or T,
K represents base G or T,
Y represents base C or T,
H represents base A or C or T,
R represents base A or G, and
D represents base A or G or T.

The conserved sequences of the first part of the *gyrB* and/or *parE* genes of all studied bacterial species were identified with this mixture of primers. It amplifies DNA from clinical samples, and has preserved sufficiently broad specificity, thus enabling the amplification of the *gyrB/parE* genes from all bacterial species causing respiratory tract infections (see Examples 6 and 7). In particular, this mixture of primers can be used to amplify the *gyrB/parE* genes of bacteria (Table 3) that are phylogenetically far from each other even in a situation where the sample includes large amounts of human DNA.

Claims

1. A diagnostic method for detecting and identifying bacterial species causing infections from a clinical sample, characterized by

a) amplifying DNA isolated from said clinical sample using a mixture of DNA primers that comprises sequences which hybridize with the sequences that originate from conserved regions of genes encoding topoisomerases, especially *gyrB/parE*, of bacterial species causing said infections, said sequences comprising sequences identified with SEQ. ID. NR: 76 and 77 or with complementary sequences thereof or functional fragments thereof,

b) contacting the amplified DNA with a desired combination of oligonucleotide probe sequences that hybridize under normal hybridization conditions with hyper-variable regions situated near said conserved regions of genes encoding topoisomerases, especially *gyrB/parE,* of bacterial species causing said infections, said sequences being bacterial species-specific under said hybridization conditions, and

c) detecting the formation of a possible hybridization complex.

2. The diagnostic method according to claim 1, characterized in that said infections causing bacterial species are bacterial species that cause respiratory tract infections.

3. The diagnostic method according to claim 1 or 2, characterized in that said hyper-variable region is the hyper-variable region of the gene encoding the *gyrB* and/or *parE* protein of a bacterial species selected from *Streptococcus pneumoniae, Streptococcus pyogenes, Chlamydia pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Escherichia coli, Moraxella catarrhalis, Legionella pneumophila*, and *Fusobacterium necrophorum*.

4. The diagnostic method according to any one of claims 1 to 3, characterized in that the length of oligonucleotide probe sequences used in step b) is 15 – 30, more preferably 20 – 30, and most preferably 21 – 25 nucleic acids.

5. The diagnostic method according to any one of claims 1 to 4, characterized in that said combination of oligonucleotide probe sequences comprises all or a portion of the sequences identified with SEQ. ID. NR: 1 to 69, and/or complementary sequences thereof, or functional fragments thereof.

6. The diagnostic method according to claim 5, characterized in that said combination of oligonucleotide probe sequences comprises all the sequences identified with SEQ ID. NR: 1 to 69.

7. The diagnostic method according to any one of claims 1 to 6, characterized in that said combination of oligonucleotide probe sequences is attached onto a solid support.

8. The diagnostic method according to claim 1, characterized in that the DNA isolated from the clinical sample in step a) is amplified using the polymerase chain reaction (PCR) and that the DNA amplified in step b) is contacted with bacterial species-specific oligonucleotide probes attached onto a solid support.

9. The diagnostic method according to claim 7 or 8, characterized in that said solid support is treated glass.

10. The diagnostic method according to claim 1, characterized in that suitably labeled nucleotides are used in the amplification of DNA isolated from a clinical sample in step a) to generate a detectable target strand.

11. The diagnostic method according to claim 10, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support, on which all bacterial species-specific oligonucleotide probes identified with SEQ. ID. NR: 1 to 69 and/or complementary sequences thereof have been attached.

12. The diagnostic method according to claim 10, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support on which specific oligonucleotide probe sequences detecting one specified bacterial species or a few specified bacterial species causing infections have been attached, said sequences being selected from sequences shown in Tables 4A and 4B and/or complementary sequences thereof.

13. The diagnostic method according to any one of claims 1 – 12, characterized in that the microarray technology is used in step c).

14. A DNA primer mixture, characterized by comprising sequences that hybridize with sequences of the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, of bacterial species that cause infections, especially bacterial species that cause respiratory tract infections, said mixture comprising sequences identified with SEQ. ID. NR: 76 and 77 and/or reversed or complementary sequences thereof or functional fragments thereof.

15. An oligonucleotide sequence useful in the diagnosis of infection causing bacterial species, characterized in that it hybridizes under normal hybridization conditions with a sequence of a hyper-variable region that is bacterial species-specific and is situated near the conserved regions of genes encoding topoisomerases, especially the *gyrB* and/or *parE* proteins, said oligonucleotide sequence being one of the sequences identified with SEQ ID. NR: 1 to 69 and/or complementary sequences thereof functional fragments thereof.

16. The combination of oligonucleotide probe sequences useful in the diagnosis of infection causing bacterial species, characterized by comprising any combination of the sequences identified with SEQ. ID. NR: 1 to 69 and/or complementary sequences thereof or functional fragments thereof.

17. The combination of oligonucleotide probes according to claim 16, characterized by comprising all of the sequences identified with SEQ. ID. NR: 1 to 69.

18. The use of the combination of oligonucleotide probes according to claim 16 or 17 for the detection, identification, or classification of infection causing bacterial species.

19. A diagnostic kit for use in the diagnosis of infection-causing bacteria, especially those causing respiratory tract infections, characterized by comprising

a) a DNA primer mixture comprising sequences that hybridize with sequences of the conserved regions of genes encoding topoisomerases, especially the gyrB and/or parE proteins, of bacterial species that cause infections, especially bacterial species that cause respiratory tract infections, said mixture comprising sequences identified with SEQ. ID. NR: 76 and 77 and/or complementary sequences thereof or functional fragments thereof.of the invention as defined above,

b) a combination of bacterial species-specific oligonucleotide probe sequences, optionally attached on a solid support, comprising any combination of the sequences identified with SEQ. ID. NR: 1 to 69 and/or reverse or complementary sequences thereof or functional fragments thereof.

c) positive and optionally negative control probe sequences, and optionally

d) reagents required in the amplification, hybridisation, purification washing, and/or detection steps.